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STABILITY OF NITROCELLULOSE TO MICROBIAL DEGRADATION

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The susceptibility of nitrocellulose to microbial attack was studied as part of a program on ball powder control technology at Badger Army Ammunition Plant, Baraboo, WI. No evidence was found to support the notion that high concentrations of ammonia may be derived from microbial attack on nitrocellulose. Nitrates, nitrites, and nitrogen gases, in addition to ammonia, were monitored. The nitrocellulose supplied by Badger was found to contain only minimal concentrations of impurities. These findings are discussed in light of other reports

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(continued) in the literature as well as the role of alkaline environments in nitrocellulose stability and subsequent susceptibility to microbial attack.

Preface

Nitrocellulose has been suggested as a possible source for ammonia contamination of receiving waters around the Badger Army Ammunition Plant, Baraboo, WI. The question of biological stability of nitrocellulose has been previously addressed by this laboratory. New efforts were initiated to study this question due to the concerns noted above.

This work was performed for the US Army Toxic and Hazardous Materials Agency (USATHAMA) under project P112.03.05, D048, W-72, 33214157000. We want to thank the personnel at Badger Army Ammunition Plant for their assistance. We also want to thank Jennifer Pierce of US Army Natick Research and Development Center for her technical assistance.

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STABILITY OF NITROCELLULOSE TO MICROBIAL DEGRADATION

INTRODUCTION

Settling pond and fresh water sediments around the Badger Army Ammunition Plant (BAAP) are contaminated with nitrocellulose. Neutralized process waters from the plant were fed into these shallow settling ponds with effluents in turn entering Gruber's Grove Bay and the Wisconsin River. Analysis of these receiving waters indicates high concentrations of ammonia, which the state of Wisconsin attributes to the decomposition of the nitrocellulose in these sediments. Ammonia concentrations in Gruber's Grove Bay were 1363 ± 1060 parts per million (ppm) and ranged from 70 ppm to 3000 ppm.[3] The state may request that these sediments be dredged in order to remove the contamination problem and return the waters to their original condition before the plant was established in 1942. The preponderance of agricultural activities on the lands around the Badger plant might also indicate agricultural run-off as a potential source of these high levels of ammonia. Subsequent sampling of upstream waters (Weigand Bay) and receiving waters (Gruber's Grove Bay) found 520 ± 199 ppm and 510 ± 224 ppm ammonia, respectively (personal communication, Mr. George Shalabi, BAAP).

Nitrocellulose (smokeless powder, gun cotton, blasting gelatin, dynamite) is a highly substituted cellulose synthesized from wood pulp or cotton and used in ball powder propellant for small arms ammunition. Nitrocellulose is also used in adhesives, varnishes, membranes, printing and pharmaceuticals. This nitrate ester is insoluble in water and should contain between 14.5% nitrogen (cellulose trinitrate) and 11.11% nitrogen (cellulose dinitrate).[6] Nitrocellulose was found not toxic to a variety of aquatic invertebrates and fish, and Environmental Protection Agency water quality criteria for solids and turbidity were deemed adequate to insure protection of the aquatic environment from nitrocellulose contamination.[3] Nitrocellulose usually enters process effluents as a suspension of very fine particles (fines).

The BAAP has been inactive since 1976, however, reactivation of the plant to meet nonmobilization small arms munitions requirements is planned. More efficient treatment systems must be utilized before the facility can be reactivated. The settling ponds used to treat process effluents in the past are insufficient to meet current regulatory discharge requirements. As part of this effort, an assessment of the potential for biological treatment of a number of the compounds present in the BAAP effluents is underway including the stability of nitrocellulose to microbial attack and the possibility of ammonia production from nitrocellulose.

MATERIALS AND METHODS

Chemicals: Nitrocellulose was provided by Olin Corp. from BAAP, Baraboo, WI, and contained 13.14% nitrogen and 26% moisture.

Sample Preparation and Analysis: Samples, 30 mL, were withdrawn and filtered through disposable Nalgene membrane filter units with a 0.2 um pore size. Nitrate, pH, oxidation-reduction and ammonia readings were recorded on a

Corning model 130 pH meter with Orion ion-specific electrodes in the case of nitrate and ammonia. A Corning pH electrode and calomel reference electrode were used to record pH. Nitrite concentrations were determined on a Perkin-Elmer Lambda 3 UV/VIS spectrophotometer using standard procedures.[1] Detection limits were 0.76 ppm (3.8 ug), 2.13 ppm (0.107 mg), and 0.06 ppm (0.3 ug) for ammonia, nitrate, and nitrite, respectively.

Head space gases were obtained with a gas-tight syringe through rubber septa on the flasks. Samples, 0.5 mL, were analyzed on a Hewlett-Packard gas chromatograph model 5880A, with a thermal conductivity detector. The injector and detector temperatures were 150°C and 275°C, respectively. Helium carrier gas flowed at 30 mL per minute through a 2.44 m by 0.32 cm stainless steel column containing Carbosieve S, 120/140 mesh. Programmed runs were initiated with an oven temperature at 35°C for 5 minutes followed by a program rate of 15°C per minute to a final temperature of 175°C for 15 minutes.

Experiment One. Simulated Lake Bottom Study: This first experiment evaluated the susceptibility of nitrocellulose to microbial attack in simulated lake bottom environments. One liter Erlenmeyer flasks contained 500 mL of media. The different media compositions included: (1) lake water at pH 6.9; (2) lake water with trace salts ($MgSO_4.7H_2O$ 500mg/L; NaCl; $CaCl_2$, 15 mg/L; $FeCl_3.6H_2O$ 10 mg/L; $CuSO_4.5H_2O$ 10 mg/L; $MnSO_4 \cdot H_2O$ 10 mg/L; $Na_2MoO_4.2H_2O$ 1 mg/L) and Difco (Detroit, MI) yeast extract, 20 mg/L at pH 6.3; (3) lake water with garden soil at pH 6.5; (4) lake water with lake sediments at pH 6.8, and (5) lake water with a mixture of lake sediment and anaerobic sludge at pH 5.0 with 0.025% sodium sulfide added to induce chemical reduction and strict anaerobic conditions. The test and control flasks were flushed with nitrogen on sampling days. For each incubation condition there was a test flask with 5% nitrocellulose and a corresponding control flask without nitrocellulose. Some of the incubation conditions were replicated three times for both test and control flasks in follow-up experiments to this initial set-up.

The sediment and sludge mixture had a moisture content of 85.5% and an organic matter content of 81.0% by ignition. The moisture content of the garden soil was 44.9% with an organic matter content of 56.3% by ignition. The sediment had a moisture content of 9.64% and an organic matter content of 1.1% by ignition. To the appropriate flasks, 65 g of soil or 150 g sediment, wet weights, were added.

Experiment Two. Stability and Impurity Study: The physical and chemical stability of nitrocellulose and the presence of chemical contaminants were evaluated in this second experiment. Flasks, 100 mL, contained 1%, 5% and 10% nitrocellulose in distilled water at pH 5.9 and 4.0 and were placed on a reciprocal shaker. Corresponding flasks containing only distilled water were run concurrently. Samples, 15 mL, were withdrawn and analyzed every four days for two weeks.

Experiment Three. Stability Under Aerobic Conditions: The third experiment was a short term study to determine the effects of aerobic biological activity on the stability of nitrocellulose. One liter Erlenmeyer flasks containing

500 mL of media were placed on a reciprocal shaker. The media consisted of: lake water, trace salts and 3% glucose at pH 7.1, lake water, fungal salts and 3% glucose at pH 5.0, distilled water with 5% malt extract at pH 5.6, and nutrient broth 4 g/L (Difco) at pH 7.0.

Test flasks contained 5% nitrocellulose and the corresponding control flasks contained no nitrocellulose. Samples, 30 mL, were withdrawn weekly or every other week.

RESULTS

Simulated Lake Bottom Study: The results of nitrate, nitrite, and ammonia analyses for this experiment are presented in Tables 1 to 3. In Table 1, monthly nitrate concentrations in control and test flasks are presented. The largest difference in nitrate readings where test (nitrocellulose) flasks were higher than the corresponding control (without nitrocellulose) flask, was 7.1 mg. This difference is not considered significant based on the concentration of nitrocellulose (25 g per test flask) in the experiment and the generally low readings recorded throughout the year-long study. Throughout the study only trace levels of nitrite were detected in both test and control systems (Table 2).

Ammonia levels are presented in Table 3, and as with the nitrate concentrations, there are few indications of higher concentrations of ammonia in the test flasks with nitrocellulose when compared with the corresponding controls. The majority of the readings were below 1 mg per flask. The largest difference in ammonia readings where test (nitrocellulose) flasks were higher than the corresponding control (without nitrocellulose) flask, was 1.5 mg.

The results from head space gas analyses are presented in Figures 1 to 9. There was no trend towards increasing or decreasing nitrogen gas concentrations. Methane, an indicator of anaerobiosis, was present in the anaerobic sediment and sludge incubation in both the control and test flasks.

Nutrient broth, 4 g/L at pH 7.0 was also looked at as a medium in this study. However, this artificially rich medium resulted in the production of high concentrations of ammonia, irregardless of the presence of nitrocellulose. A separate study was conducted with nutrient broth which confirmed the fact that there were no significant differences in the ammonia, nitrites or nitrates produced from this medium between test samples (with 5% nitrocellulose) and controls (without nitrocellulose).

TABLE 1. Simulated Lake Bottom Study; Nitrate Concentrations

Incubation Conditions	CONCENTRATION NO_2 (mg per flask) Months												
	0	1	2	3	4	5	6	7	8	9	10	11	12
1. Lake Water (ph 6.9)													
Control[a]	0	0.1	0	0	0	0	0	0	0	0	-[c]	-	0
Nitrocellulose[b]	0.6	1.9	2.6	3.5	3.2	0.2	2.0	0	0.1	0	-	-	0
2. Lake Water with Trace Salts & Yeast Extract													
Control	0	0	0	0	0	0	0	0	0	0	-	-	0
Nitrocellulose	1.1	1.7	0	0	0	0	0	0	0	0	-	-	0
3. Lake Water & Soil													
Control	0.2	0	0	0	0	0	0	0	0.1	0	-	-	0
Nitrocellulose	0.6	0	0	0	0	0	0	0	0.1	0	-	-	0
4. Lake Water & Sediment													
Control	0.1	0	0	0	0	0	0	0	0	0	-	-	0
Nitrocellulose	0.6	0	0	0	0	0	0	0	0.1	0	-	-	0
5. Lake Water, Sludge & Sediment (strict anaerobic conditions)													
Control	0	0	-	0	0	0	0	-	0	0	0	0	0
Nitrocellulose	0	0	-	0	0.5	0	0	-	0	0	0	0	0

[a]Specific incubation conditions without nitrocellulose added

[b]5% Nitrocellulose in corresponding test flask

[c]No data

TABLE 2. Simulated Lake Bottom Study; Nitrite Concentrations

CONCENTRATION NO_3 (mg per flask)

Incubation Conditions	Months 0	1	2	3	4	5	6	7	8	9	10	11	12
1. Lake Water (ph 6.9)													
Control[a]	4.3	1.1	3.3	1.1	5.2	5.6	0.9	0.6	0.5	0.6	-[c]	-	0.8
Nitrocellulose[b]	6.1	2.3	3.5	2.4	8.7	8.4	2.7	2.7	2.6	2.2	-	-	7.2
2. Lake Water with Trace Salts & Yeast Extract													
Control	4.2	1.4	2.0	1.1	5.5	4.8	1.1	0.6	0.7	0.8	-	-	3.2
Nitrocellulose	4.1	2.4	7.1	3.9	11.1	7.6	3.8	2.7	2.2	1.7	-	-	4.5
3. Lake Water & Soil													
Control	91.7	32.7	6.4	4.1	23.9	32.4	2.1	1.2	1.0	2.1	-	-	2.0
Nitrocellulose	41.4	23.3	5.4	3.6	20.8	25.2	1.7	1.0	0.8	1.7	-	-	2.0
4. Lake Water & Sediment													
Control	3.5	5.6	6.0	4.5	11.6	7.9	1.0	0.4	0.7	0.9	-	-	1.1
Nitrocellulose	4.2	5.5	2.1	1.5	4.9	4.6	0.8	0.3	0.3	0.7	-	-	0.8
5. Lake Water, Sludge & Sediment (strict anaerobic conditions)													
Control	1.3	1.7	-	8.2	9.1	8.0	2.8	-	2.2	1.4	2.0	1.5	3.6
Nitrocellulose	1.4	4.5	-	8.1	7.1	4.6	1.8	-	1.7	0.9	9.1	0.7	1.7

[a]Specific incubation condition without nitrocellulose added

[b]5% Nitrocellulose in corresponding test flask

[c]No data

TABLE 3. Simulated Lake Bottom Study; Ammonia Concentrations

CONCENTRATION NH_3 (mg per flask)

Incubation Conditions	Months 0	1	2	3	4	5	6	7	8	9	10	11	12
1. Lake Water (ph 6.9)													
Control[a]	0.2	0.2	0.3	0.1	0.2	0.1	0.1	0.1	0.2	0.2	-[c]	-	0.2
Nitrocellulose[b]	0.3	0.5	0.5	0.0	0.6	0.4	0.4	0.3	0.3	0.6	-	-	0.2
2. Lake Water with Trace Salts & Yeast Extract													
Control	0.2	0.8	2.6	0.7	0.5	0.3	0.2	0.2	0.2	0.0	-	-	0.1
Nitrocellulose	0.2	0.9	0.9	1.1	0.4	0.1	0.1	0.1	0.1	0.1	-	-	0.1
3. Lake Water & Soil													
Control	0.4	0.4	0.6	0.5	0.5	0.7	0.5	0.6	0.7	0.3	-	-	0.2
Nitrocellulose	0.4	0.2	0.2	0.2	0.3	0.3	0.1	0.1	0.5	0.1	-	-	0.1
4. Lake Water & Sediments													
Controls	0.2	0.2	0.2	0.2	0.4	0.1	0.1	0.1	0.7	0.2	-	-	0.1
Nitrocellulose	0.4	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	-	-	0.1
5. Lake Water, Sludge & Sediment (strict anaerobic conditions)													
Control	9.9	9.4	-	10.8	10.0	7.0	4.0	-	4.8	2.7	1.8	0.3	0.7
Nitrocellulose	11.4	3.6	-	11.2	3.6	0.2	0.1	-	0.2	0.3	0.2	0.2	0.5

[a]Specific incubation condition without nitrocellulose added

[b]5% Nitrocellulose in corresponding test flask

[c]No data

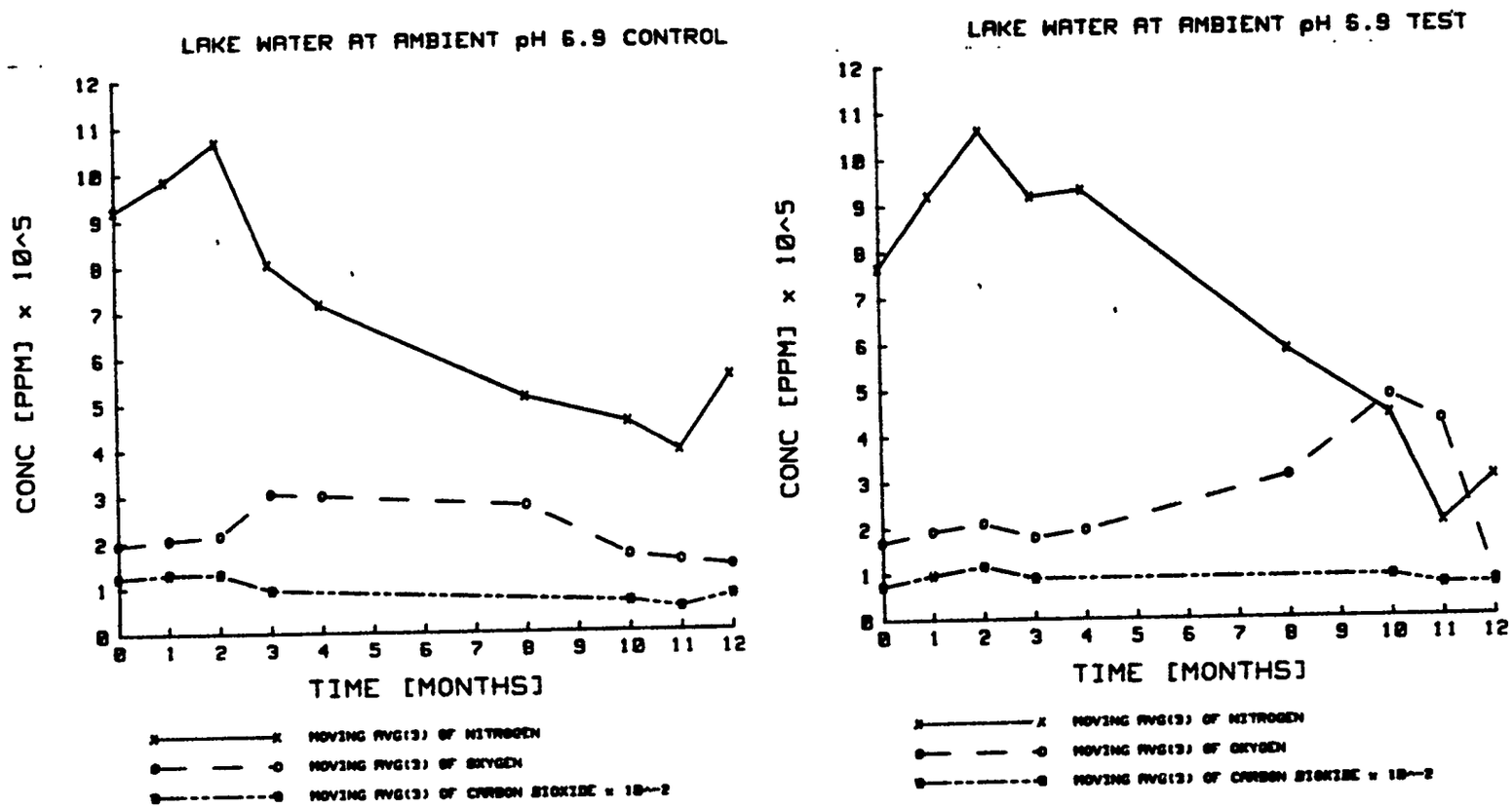

Figure 1. Lake water control (left) and test flask (right) head space gas analysis.

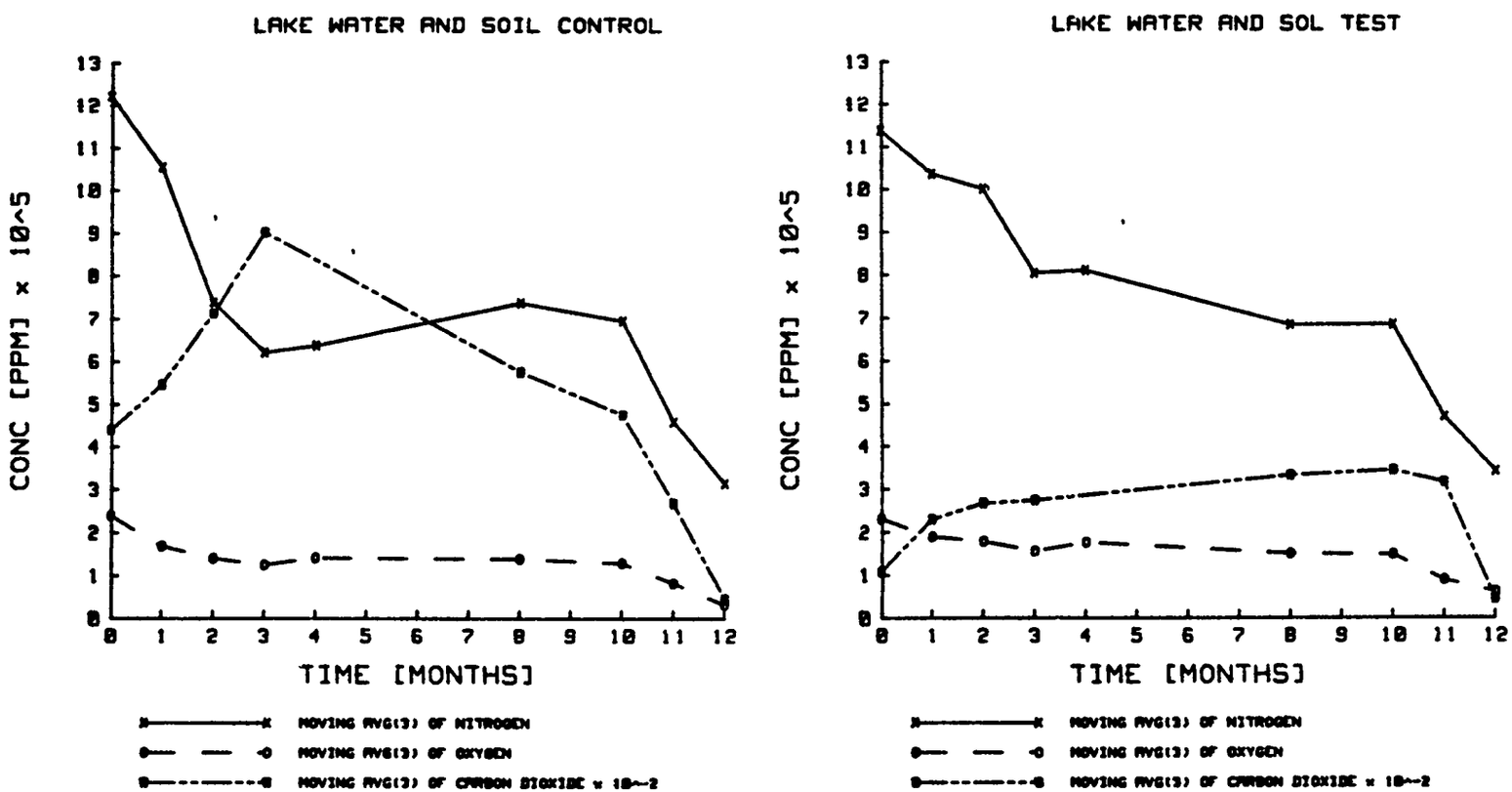

Figure 2. Lake water and soil control flask (left) and test flask (right) head space gas analysis. Nitrous oxide was present in the first month of testing in the control and test flask head space of 6.2×10^3 and 4.2×10^3 ppm N_2 respectively.

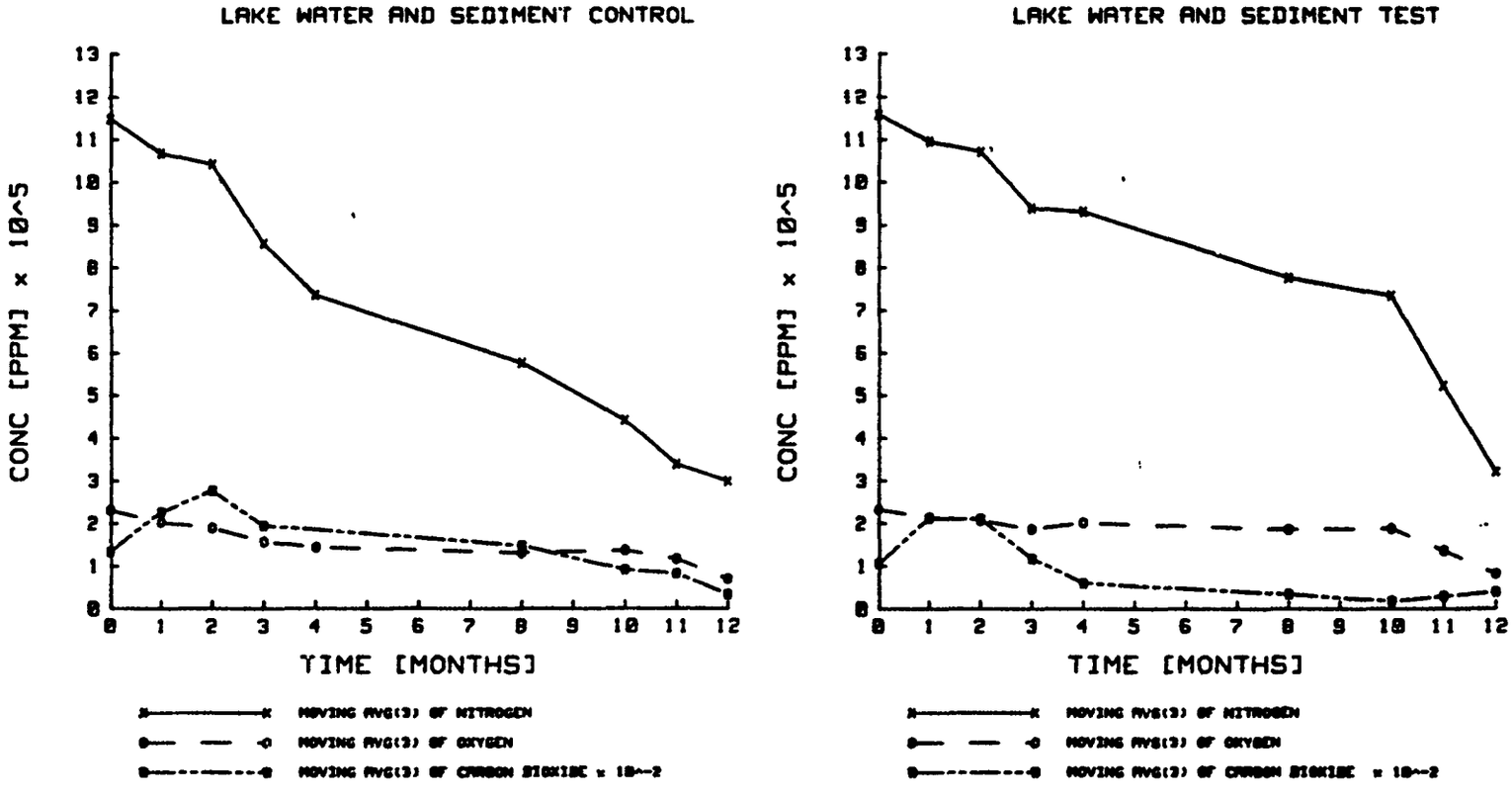

Figure 3. Lake water and sediment control flask (left) and test flask (right) head space gas analysis.

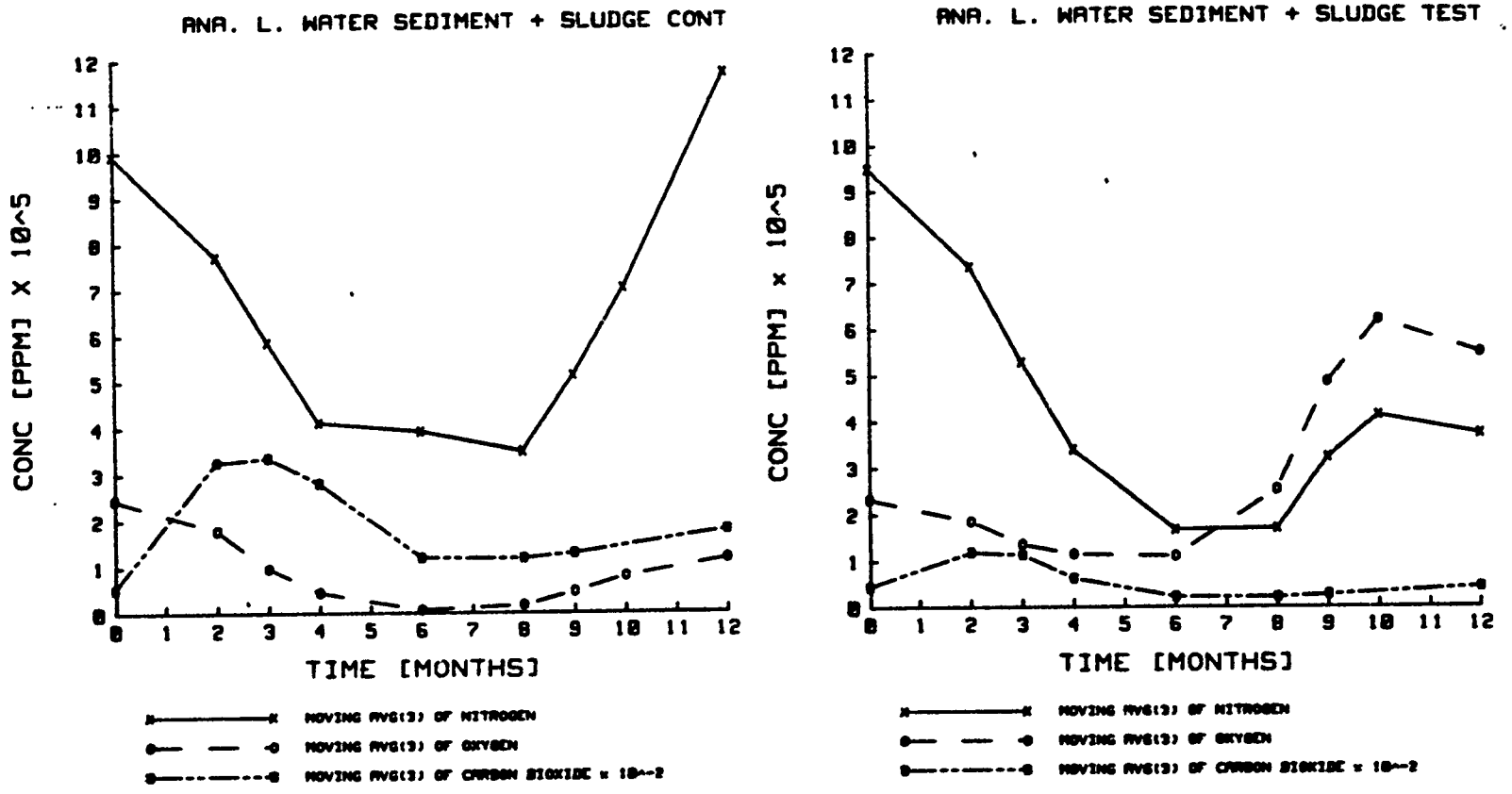

Figure 4. Lake water, sludge and sediment control flask (left) and test flask (right) head space gas analysis. Methane was present in the control flask head space during the sixth through twelfth months of testing at 9390, 140, 50, 800 and 520 ppm, respectively, and in the test flask head space during the twelfth month at 840 ppm.

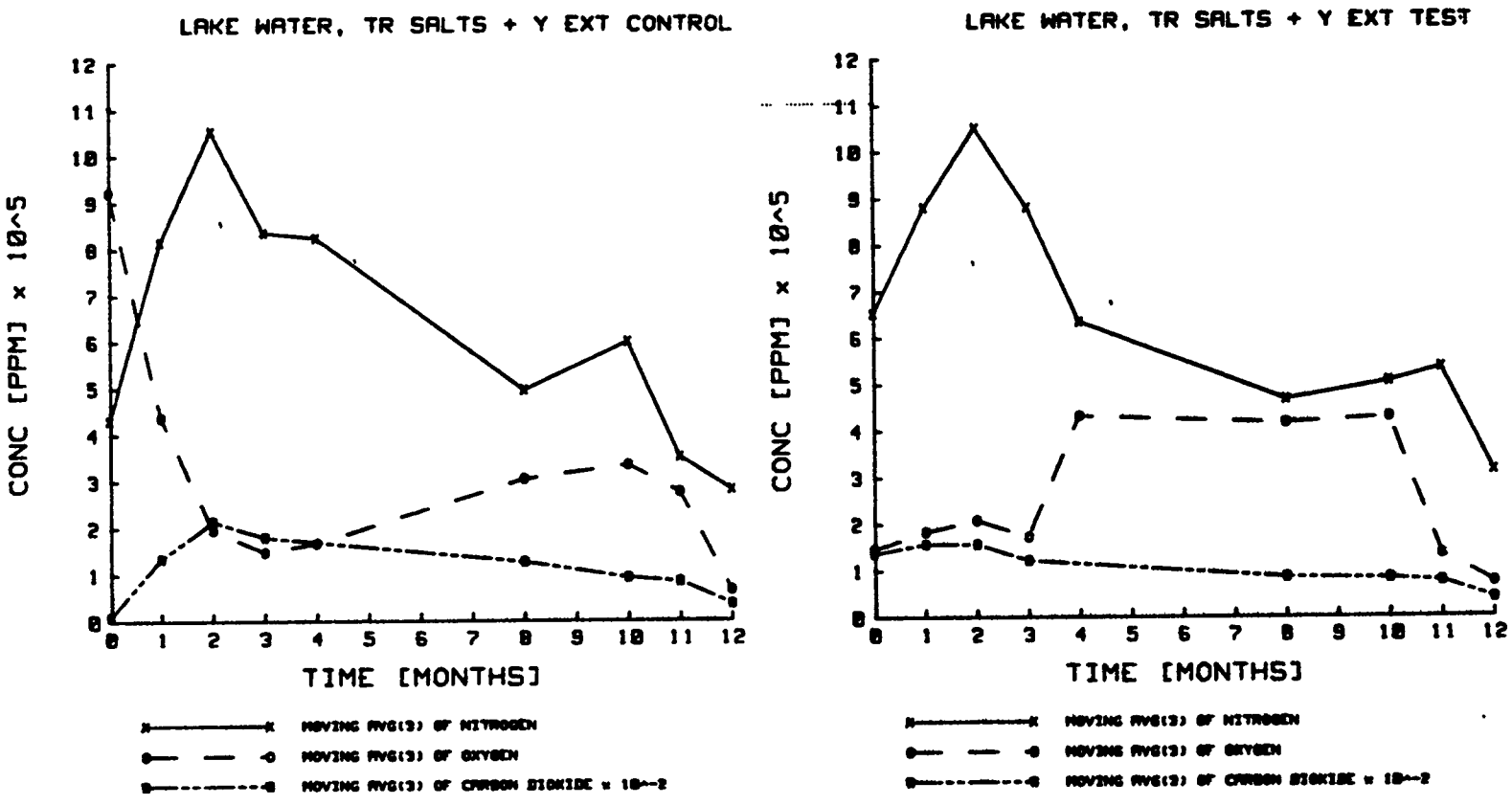

Figure 5. Lake water, trace salts, yeast extract and 3% glucose control flask (left) and test flask (right) head space gas analysis.

Data on pH and oxidation potential of control and test flasks are presented in Table 4. The anaerobic sediment study control and test flasks showed an increase in pH during the study. The data on oxidation-reduction show the systems became more aerobic during the year with the exception of the lake sediment and sludge study.

Stability and Impurity Study: Table 5 illustrates the data on pH, which increases rapidly after the start of the experiment in flasks containing 5% and 10% nitrocellulose initiated at pH 4.0. In Table 6 the cumulative percent release of nitrate from nitrocellulose in buffered distilled water, as a measure of residual nitrate impurities contaminating the nitrocellulose, was at most 0.005% throughout the experiment. The cumulative maximum was approximately 0.06 mg nitrate throughout the experiment.

Stability Under Aerobic Conditions: The results of analysis for experiment 3 are presented in Table 7. High nitrate concentrations were found in the artificially rich malt extract incubation test flask although there was no significantly higher nitrite on ammonia concentrations.

Head space gas analysis revealed oxygen, nitrogen, carbon dioxide and water vapor in all incubations. Nitrous oxide was detected on day 12 in all head space samples. Hydrogen ion concentrations increased in all flasks during the study, with lake water fungal salts and 3% glucose showing a decrease in pH from a pH of 7.1 to pH 3.1 in the control flask.

TABLE 4. Average Hydrogen Ion Concentration and Oxidation-Reduction Potentials during the 12 Month Testing Period

Incubation Conditions	pH $\bar{x} \pm 1$ S.D.	Redox[1] (millivolts) $\bar{x} \pm 1$ S.D.
1. Lake Water (pH 6.9)		
Control	7.6 ± 0.67	121.3 ± 51.8
Nitrocellulose[2]	7.0 ± 0.39	150.4 ± 44.9
2. Lake Water with Trace Salts & Yeast Extract		
Control	6.9 ± 0.36	209.2 ± 38.6
Nitrocellulose	6.8 ± 0.40	199.0 ± 41.8
3. Lake Water + Soil		
Control	6.8 ± 0.16	99.0 ± 82.3
Nitrocellulose	7.2 ± 0.44	109.3 ± 52.9
4. Lake Water + Sediment		
Control	6.8 ± 0.23	103.8 ± 66.4
Nitrocellulose	7.6 ± 0.80	53.9 ± 107.1
5. Lake Water, Sludge + Sediment (anaerobic conditions)		
Control	6.2 ± 0.40	-45.9 ± 191.8
Nitrocellulose	6.1 ± 0.30	-1.5 ± 221.7

[1]Oxidation-reduction potentials recorded from the third month of testing.

[2]5% Nitrocellulose in test flask.

TABLE 5. Hydrogen Ion Concentration

Contents	Initial pH	Time (days) 0.17	4	8	11	15
Control dH_2O	5.9	6.2	6.3	6.4	6.7	6.1
	4.0	4.1	4.0	4.0	4.0	4.0
1% Cellulose Nitrate	5.9	6.5	6.4	6.4	6.3	6.3
	4.0	4.1	4.1	4.3	4.2	4.3
5% Cellulose Nitrate	5.9	6.8	6.8	6.8	6.9	6.6
	4.0	5.7	6.2	6.3	6.3	6.2
10% Cellulose Nitrate	5.9	6.6	6.6	6.6	6.6	6.5
	4.0	6.7	6.9	6.8	6.8	6.7

TABLE 6. Nitrate - Percent Release - Cumulative

Contents	Initial pH	Time (days) 0	0.17	4	8	11	15
1% Cellulose Nitrate	5.9	.001	.001	.000	.002	.002	.002
	4.0	.002	.001	.001	.002	.001	.001
5% Cellulose Nitrate	5.9	.001	.001	.002	.001	.005	.003
	4.0	.004	.003	.005	.005	.005	.004
10% Cellulose Nitrate	5.9	.002	.002	.003	.002	.002	.002
	4.0	.001	.001	.002	.002	.002	.002

TABLE 7. Decomposition of Nitrocellulose in Simulated Aerobic Lake Bottom Experiments

Incubation Conditions	Nitrate (mg)[1] $\bar{x} \pm 1$ S.D.	Nitrite (mg) $\bar{x} \pm 1$ S.D.	Ammonia (mg) $\bar{x} \pm 1$ S.D.	pH	Eh (millivolts)
1. Lake Water, Trace Salts, + 3% Glucose					
Control	21.6 ± 40.7	N.D.[2]	0.3 ± 0.1[3]	3.1 ± 1.2	115.1 ± 61.8
Nitrocellulose[1]	3.6 ± 1.4	0.2 ± 0.4	0.3 ± 0.1[3]	3.2 ± 1.5	109.2 ± 66.8
2. Lake Water, Fungal Salts + 3% Glucose					
Control	2.2 ± 0.9	N.D.	0.3 ± 0.0	4.6 ± 1.6	200.8 ± 23.8
Nitrocellulose	4.6 ± 1.7	0.1 ± 0.3	0.2 ± 0.1	4.9 ± 1.2	199.8 ± 24.9
3. Nutrient broth 50%					
Control	64.2 ± 37.6	N.D.	244.7 ± 150.4	6.9 ± 0.3	-108.8 ± 145.1
Nitrocellulose	55.6 ± 34.3	0.1 ± 0.2	215.6 ± 127.1	6.9 ± 0.3	-55.5 ± 108.6
4. Malt 5%					
Control	6.0 ± 2.6	0.6 ± 1.3	0.3 ± 0.6	4.0 ± 0.9	54.9 ± 73.2
Nitrocellulose	172.0 ± 165.2	0.1 ± 0.2	0.5 ± 0.7	3.6 ± 1.3	74.6 ± 63.4

[1]5% Nitrocellulose in test flask.

[2]Not detected

[3]Data reported as days 9-45.

DISCUSSION

The cumulative results from all three experiments indicate that nitrocellulose is not susceptible to microbial attack, as reflected by nitrate, nitrite, ammonia and N-gas production. In instances where nitrate, nitrite, or ammonia concentrations were higher in test (nitrocellulose) than control (without nitrocellulose) flasks, the magnitude of these differences was insignificant in light of the actual levels of ammonia contamination in the field.

Overall, vigorous aerobic conditions, as studied in the aerobic stability study, do not magnify the static conditions to any great extent. The nitrogen products formed are fairly consistent in concentration. Both static and reciprocal shaker experiments show no significant ammonia production due to the biodegradation of nitrocellulose.

Brodman and Devine[2] reported significant nitrate release due to hydrolysis of nitrocellulose due to the activity of microorganisms. When adjusted for controls, their data report a cumulative percent release of 0.203% nitrate at a 1% nitrocellulose concentration. In our stability and impurity study we showed a 0.005% cumulative release of nitrate with a five-fold higher initial concentration of nitrocellulose. This large discrepancy in findings may be due to a higher degree of contamination of the nitrocellulose used in the work of Brodman and Devine, but it is not due to microbial susceptibility of the substrate as was claimed by Brodman and Devine.

Ammonia production from nitrocellulose seems unlikely in light of the results presented in this report. Also, the existence of a nitrate esterase has never been reported in the literature, and it is well known that even a small percentage of substitution of the cellulose renders the compound resistant to attack by cellulolytic organisms. However, if the nitrocellulose containing process waters were exposed to alkaline conditions at some point during their production, then partial hydrolysis may occur, and thus the hydrolyzed nitrocellulose could then be susceptible to microbial attack.[7] This could also be the case if the lake bottom where the nitrocellulose fines have accumulated are present in an alkaline environment. The extent of these possibilities at BAAP are unknown. Since significant levels of ammonia contamination have also been found upstream from the plant, agricultural run-off may be the more likely source of this contamination.

At Radford Army Ammunition Plant (Radford, VA), a study was conducted to evaluate nitrocellulose fines when limed nitrocellulose was placed in a landfill environment. In this alkaline situation low ammonia concentrations were found.[4] As shown by this study and stated by Urbanski (1965), ammonia can be produced by hydrolysis of nitrocellulose in an alkaline environment.[6]

Contamination of nitrocellulose with residual acids or other impurities could also potentially lead to release of ammonia or nitrate from process water. However, results from our stability and impurity study strongly indicate contamination of BAAP nitrocellulose, as supplied, was not a problem.

In general, the results from the above studies provide little evidence to support the notion that biological attack of nitrocellulose will result in the production of significant concentrations of ammonia, nitrate, nitrite, or nitrogen gases. No evidence for high concentrations of impurities in nitrocellulose from Badger Army Ammunition Plant was found. Results from a series of studies, including one year simulated lake bottom incubations, indicate that nitrocellulose is not susceptible to biological attack. Nitrocellulose is an unlikely source for ammonia contamination of waters around Badger Army Ammunition Plant.

CONCLUSIONS

The cumulative results from these studies indicate that nitrocellulose is not susceptible to microbial attack. No evidence was found for high concentrations of nitrogen impurities in nitrocellulose from Badger Army Ammunition Plant. The results of these studies demonstrate that nitrocellulose is stable to biological attack and thererore an unlikely source for ammonia contamination of waters around Badger.

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